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Considerations on microbial electrosynthesis

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Abstract

Extracellular electron transfer has in one decade emerged from an environmental phenomenon to an industrial process driver. On the one hand, electron transfer towards anodes leads to production of power or chemicals such as hydrogen, caustic soda and hydrogen peroxide. On the other hand, electron transfer from cathodes enables bioremediation and bioproduction. While the microbiology of extracellular electron transfer is increasingly understood, bringing the processes to practice requires a number of considerations that are both operational and technical. In this manuscript we investigate the key applied aspects related to electricity driven bioproduction, including biofilm development, reactor and electrode design, substrate fluxes, surface chemistry, hydrodynamics and electrochemistry, and finally end product removal/toxicity. Each of these aspects will be critical for the full exploitation of the intriguing physiological feat extracellular electron transfer is today.

Key words

Bioelectrochemical system, bioproduction, extracellular electron transfer, microbial electrosynthesis

Introduction

The capability of microorganisms to exchange electrons with solid substrates has particularly in the past decade led to a new technology field: microbial electrochemistry. Microorganisms that use solid-state electrodes as electron acceptors are used to oxidize wastewater organics [1] and correct the redox balance in fermentations [2]. This leads to bioenergy production, better fermentation or energy efficient electron supply for secondary processes. Similarly, microorganisms using solid-state electrodes as electron donors are used to remediate sediments [3] and water [4], fix CO₂ into useful products [5] and redirect fermentation pathways [6]. The use of electrical current to drive a production process, whether it is oxidative or reductive and whether it leads to fixation of CO₂ or conversion of organics is called microbial electrosynthesis [7, 8]. One can summarize the key benefit of electrode driven microbial conversions as the ability to separate the oxidation from the reduction reaction leading to stronger oxidative/reductive conditions or more controlled aqueous chemistry. A key example of the latter is the production of inorganic chemicals such as caustic [9] and peroxide [10] driven by microbial organics oxidation.

The mechanisms by which microorganisms transfer electrons in and out of the cell are increasingly understood. Microorganisms can use soluble shuttles for electron transport [11-13], but can also transfer electrons in a more direct way via cell wall bound complexes, typically cytochromes [14]. These can function in conjunction with conductive appendages called nanowires, that enable electron transport through a solid state protein based matrix [15-17]. For the purpose of this manuscript, it is important to note that microorganisms can thus be (i) in a suspension (planktonic) where they reduce/oxidize electron shuttles, or (ii) they can form a biofilm and maintain a continuous electrical link with the electrode. A variation on these possibilities is a “capacitive” metabolism where cells charge up their terminal complexes while migrating and discharge upon contacting an electrode or mineral particle [18].

Upon assuming that microorganisms are available that can rapidly use electrical current to drive production processes, it is important to note that microbial electrosynthesis, as a *surface* based technology, still faces a considerable number of technical and operational issues. In this manuscript we consider a number of these key issues identified at present, both for CO₂ and organics based microbial electrosynthesis.

Basic economics: What is worth producing starting from CO₂?

There are several advantages of using CO₂ as a substrate for bioproduction such as unlimited availability (atmosphere, waste gas), land-independence and limited toxicity to the microorganisms (Table 1). To highlight the economical driving force behind the MES concept, we considered a case where a 50 m² BES system works at 20 A m⁻² current density (projected on the membrane electrode assembly) at 1 V operational, resulting in a system producing biochemicals at 1000 A. Starting from CO₂ as a substrate and assuming 80% product efficiency, a range of products and their value according to 2012 market prices were calculated in Table 2. Furthermore, an energy investment at current prices of €0.1 per kWh can be assumed, implying daily power costs of ~ € 2.4 not including other operational inputs.

The permissible system cost stresses the challenge existing for electricity driven bioproduction. It is a safe assumption that for an electrochemical system the total system cost is easily 3 times the reactor cost, due to a range of costs such as engineering design, peripherals, civil structures and piping. On such an assumption, one can see that the acceptable cost to produce a functional bioelectrochemical reactor on a simple 3-year payback time is €17 to 196 m⁻² installed, depending on the product value (Table 2).

What is worth producing starting from organics?

A key disadvantage of using CO₂ as a substrate for product formation is the large electron requirement for the synthesis of organic compounds [7]. Therefore, the use of more reduced substrates, i.e. organics (glucose, acetate, butyrate, lactate...) can be an interesting alternative as it significantly lowers the current and thus power demand of the production process. In most cases obtaining pure substrate as starting point will be too expensive, considering the initial refining required. However, there is a considerable supply of waste organics with limited value such as glycerol and fatty acids containing streams. Converting glycerol to 1,3-propanediol delivers a considerable value increase. Likewise, the elongation of short chain fatty acids such as acetate to caproate, which is more straightforward to capture, provides a similar value uplift (Table 2). It appears that at least on the short term organics based conversions can deliver higher return on investment. Furthermore, the acceptable system cost is a factor 10 higher (232-1684 € m⁻² installed) compared to CO₂ derived product formation (Table 2).

Metabolic aspects

Microbial electrosynthesis relies on reducing equivalents being delivered by an electrode to a microorganism to drive an anabolic reaction in the cathode compartment, or it relies on the discharge of excess reducing equivalents towards an electrode in the anode compartment. Here the focus will be on the processes in the cathode. Growth of microorganisms using a cathode as electron donor has been very challenging thus far. One can however argue that growth is not strictly required. Indeed, upon omitting growth factors, the microbes may act as a true catalyst i.e. only offering their (exposed) enzymes for catalysis of a certain reaction. Various studies have shown the production of H₂ [19, 20] or CH₄ [21, 22] or dechlorination [23] reactions, but growth solely dependent on current has not yet been unequivocally shown. Without growth, the microorganism only functions as a 'scaffold' for the enzymes of interest, although regular "maintenance" of the organisms may be required. This line of thought is suggesting an optimized feast and famine regime to maximize biofilm based microbial electrosynthetic production.

During electrosynthesis, electrons are made directly available at the cathode. These electrons need to be linked up with the electron transport machinery of the microbial cell. This is most likely achieved by direct electron transfer with cytochromes and (de)hydrogenases in the outer membrane in a biofilm process. Indirect transfer can also occur when mediators are used. In that case, the electrons need to be transported on a carrier such as H₂, or a redox mediator. In this context, Rosenbaum and co-workers have reviewed various cathodic electron transfer mechanisms based on anodic electron transfer [24]. However, considerable work still needs to be done to elucidate the actual mechanisms that take place.

Energy conservation for the biocatalyst

Next to the mechanisms, also the thermodynamic value chain needs to be taken into account. Taking the example of i.e. *Thiobacillus ferrooxydans* or other chemolithoautotrophic organisms, does not add much to our understanding of energy conservation for the biocatalyst since in the context of microbial electrosynthesis the final electron acceptor has a low redox potential (e.g. HCO₃⁻/HCOO⁻ E⁰' = -406 mV). In a *Thiobacillus* like metabolism energy can be gained from the electrons that are obtained from reduced mineral species since almost the whole electron transport chain can be utilized towards a high potential electron acceptor (NO₃⁻ or O₂), whilst this is not the case during

MES. Whether energy can be conserved by the microorganism depends on the 'length' of the electron transport chain that can be used and the energy conserving mechanisms that are present.

Energy carriers

Electrons that enter at the cytochromes and hydrogenases need to be connected to the quinone pool in the inner membrane, which in turn is connected to the NAD^+/NADH pool of the cell.

Microorganisms maintain a high ratio of NAD^+/NADH for catabolic purposes. The NAD^+ is used to receive reducing equivalents from reduced substrates during normal metabolism. NADH produced by an electric current can alter the NAD^+/NADH ratio and thus change the redox status of the cell. By this means, one can be enabled to interfere with the redox balance of a cell and possibly steer metabolic processes.

NADH cannot be used for all anabolic reactions, which is an important consideration when employing *de novo* electrosynthesis from CO_2 and from substrate organics. In some crucial steps NADPH needs to be present, therefore, NADH needs to be transformed into NADPH, ensuring a low ratio of $\text{NADP}^+/\text{NADPH}$ for anabolic purposes. This transformation can for example be achieved by a nicotinamide nucleotide transhydrogenase (NNT) [25]. This is an enzyme that catalyses the transfer of a hydride group (H^-) from NADH to NADP^+ . NNT can only function by means of a proton gradient across the cell membrane. Considering electrosynthesis, where NADH is most likely generated by means of a reverse electron transport chain, it is difficult to generate a proton motive force as protons or other cations will enter the cell to maintain electroneutrality. A benefit of this is that ATPases present in the cell membrane will function in the reverse direction i.e. instead of being an ATP based proton efflux system, the ATPase can function as the port of entry for protons and thus creating ATP in the process as shown by Pandit et al. in their *in silico* metabolic model [26].

De novo electrosynthesis from CO_2

CO_2 fixation can occur by means of three main pathways i.e. the Calvin cycle, a reductive TCA-cycle and the Wood/Ljungdahl pathway (reductive acetyl-CoA pathway). The latter process is linear as opposed to the former two [27]. Considering homo-acetogenic metabolism as a model for *de novo* microbial electrosynthesis of organic compounds, there are, up till now, three energy and carbon conserving mechanisms identified between all known homoacetogens.

- i) formation of a H^+ -gradient over the cell membrane by means of cytochromes and quinones and subsequent ATP formation by means of a H^+ -dependant ATPase (Example: *M. thermoacetica*) [28],
- ii) formation of a Na^+ gradient and ATP generation by means of a Na^+ -dependant ATPase (Example: *A. woodii*) [29],
- iii) formation of a H^+ - gradient via an RNF complex and ATP generation by means of a H^+ -dependant ATPase (Example: *C. kluyveri*, *C. ljungdahlii*) [30].

The above-mentioned pathways indicate that it is necessary to deeply understand the metabolic features of the catalyst of choice. Pumping electrons into an acetogen by means of hydrogen is possible through the action of hydrogenases with the formation of NADH. When NADPH (or any other electron donor; FADH or ferredoxin) is required to fuel the initial reduction of CO_2 to formate, a NNT might be required [31]. NNT is dependent on a H^+ -gradient, which might also explain why next to chemi-osmotic coupling dependant on Na^+ , till date, no MES has been achieved with *A. woodii* [5].

Operational bottlenecks

MES mainly exists conceptually as only few reports exist demonstrating the proof of concept of bioelectrochemical product formation at laboratory scale [6, 32, 5, 33]. Hence, the robustness and efficiency of such a bioelectrochemical production process still remains to be demonstrated at realistic scale, thereby producing biochemicals at rates and concentrations needed. For the latter, key operational bottlenecks have to be resolved prior to implementation.

Creating a compatible biofilm-electrode interface

Little is known about the impact of electrode surface properties on colonization and activity, and limited biocatalysts are known that can successfully grow on polarized electrodes, especially cathodes. The topography of the electrode determines cell adherence, biofilm architecture and electron transfer. A high surface to volume ratio is essential to provide a large interface between electrode and biofilm as well as decrease current density, which in turn decreases activation losses.

Currently, graphite-based materials and carbon nanotubes (CNTs) are the most commonly used electrodes [34]. However, these are generally considered to be too expensive for large-scale applications. In this respect, Xie and co-workers developed a low-cost graphene sponge electrode of which the capital cost (estimated at €3 m⁻²) is an order of magnitude lower compared to CNTs and most commercially available graphite electrodes [34]. The lower conductivity of graphene-based materials compared to graphite (ca. 2 orders of magnitude) will require integration of a current collector, however this will be necessary for any larger scale system unless executed as bipolar stack.

The surface chemistry of an electrode could be altered by the immobilization of charged molecules, but is yet a poorly investigated approach. It has been shown that increasing the positive charge of the carbon surface through ammonia gas treatment increases the power production [35]. Likewise, it has been demonstrated that the introduction of positively charged groups on the anode surface doubled the power output from 60 to 120 mW m⁻², and the formation of a dense homogenous biofilm was observed [36]. However, these kinds of surface modifications remain to be demonstrated for polarized cathodes. The latter are negatively charged as well as the bacterial outer cell envelope [37], resulting in a repulsive effect that potentially impedes cell adherence and thus biofilm development.

Keeping the biocathode alive

Some of the growth concerns were addressed previously, another important aspect inherent to an electrochemical cell is charge neutrality. To balance the electron flow, protons are the preferred positive charge transporter to avoid pH gradients. However, if the proton concentration in the anode is low (typically pH 7), other cations such as sodium or potassium become the dominant charge transporter [38, 39], resulting in acidified anodes and alkaline cathodes. Localized high pH at the cathode biofilm interface can lead to loss of proton motive force [40], thereby hampering the energy metabolism of the biocatalyst. To date, the latter is generally resolved by applying high buffer concentrations, which is unrealistic from both an economical and environmental point of view. A possible though poorly investigated alternative approach would be by applying microorganisms that rely on sodium motive force for their energy metabolism [41], as mentioned above for the model acetogen *A. woodii*.

A second solution is providing a non-limiting diffusive proton flux to the cathode biofilm by keeping the bulk pH in the cathode low. Maintaining a pH of not higher than 8 at the cathode surface requires

a bulk pH of 2.1-3.2 in order to obtain current densities in the order of $10\text{-}20\text{ A m}^{-2}$ according to a simple diffusive model (Fig. 1). The latter could be feasible by operating the anode at sufficient low pH. Otherwise, providing sufficient electrode surface area can significantly lower the required pH gradient. Furthermore, also the thickness of the laminar boundary layer plays an important role (Fig. 1), and stresses the need for sufficient mixing and optimisation of the electrode-biofilm topography.

Remaining selective towards the desired end product

From an economic perspective, it is important to maximize the recovery of the delivered electrons into the desired products. Depending on the choice of the biocatalyst, that is a pure or mixed culture, different strategies can be envisaged to optimize this process. In case pure cultures are used, the metabolism can be streamlined towards specificity but typically this approach requires batch production processes. Mixed populations are highly suitable in case waste organics are fed to the BES, however both end product toxicity and competing reactions can decrease the process effectiveness. Proper operational conditions such as low pH and high carboxylate content can alleviate some of the side reactions. Periodic introduction of specific inhibitors may further decrease competition of methanogens and also sulphate reducers.

Product inhibition and recovery

Generally, microorganisms are sensitive to high concentrations of their excreted products [42]. Product inhibition and toxicity effects can severely impede the activity of the biocatalyst. Either an integrated product separation and recovery technology needs to be implemented or the microorganisms need to be engineered to higher resistance. The latter is challenging as endowing microbes tolerance is not a matter of single gene modulation, but rather global transcription machinery engineering [43]. Whatever the envisaged strategy might be, it will only be successful if the required product titer can be reached. The latter is an important cost determinant as it affects the downstream processing cost as well as the entire processing plant footprint[44].

Conclusion: One technology, multiple outputs

The key advantage of bioelectrochemical technology is the ability to generate multiple products and/or services. Indeed, while treating wastewater at an anode one can produce a pure product at the cathode. Or, one can produce an anionic chemical at the cathode and subsequently harvest the product at the anode. Considering the cost of the production process and the value of the products generated, it appears that this will be essential (but doable) to reach a viable technology. From an operational perspective, many challenges remain due to the fact that thus far the field has only addressed the basic microbiology and engineering aspects. Developing low cost, high surface area and well-conductive electrode materials will address many of the issues existing today besides the development or selection of the appropriate biocatalysts. At a higher level, overall system architecture will need to address the fact that the catalyst in this case is voluminous and developing over time. Given an appropriate architecture and peripheral setting, MES can be a game-changing approach towards production of commodity and fine chemicals, independent of fossil fuel input.

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Tables

Table 1. A comparison between CO₂ and substrate organics as substrate for microbial electrosynthesis [8].

CO ₂	Substrate organics
<ul style="list-style-type: none"> + Available in excess in the atmosphere, seawater and in solid minerals - Low atmospheric concentrations hamper CO₂ flux into solution per unit land surface + CO₂ supply to reactor medium may provide buffering capacity - High number of electrons required for product formation as CO₂ is fully oxidized - Autotrophic growth and fixation requires energy investment by cell to activate e.g. Wood-Ljungdahl pathway + Complete or nearly complete independence of arable land - Nutrient requirement for biocatalyst growth + CO₂ removed from atmosphere provides positive impact on greenhouse gas budget (depending on electricity source and net sequestration) + CO₂ uptake by the cell does not require energy investment 	<ul style="list-style-type: none"> - Availability depends on the location and may vary depending on the size of plant or supply + High solubility of most substrate organics facilitates dosing +/- Depending on the substrate, the resulting pH upon addition may be unfavourable for bioproduction (e.g. butyrate) + The substrate is already partially reduced (containing considerable electrons), hence limited electrons needed for bioproduction + Heterotrophic growth can be achieved on substrate organics +/- May require arable land in case high quality substrate is required - Nutrient requirement for biocatalyst growth + Waste derived organics have a negative value hence processing delivers a net benefit +/- Depending on the substrate, energy may be required for transport, phosphorylation or activation of the substrate

Table 2. Production quantities for diverse chemicals assuming 1000 A current for 1 day converted at 80% efficiency to product. Product values were taken from www.icis.com/chemicalprices and www.alibaba.com/showroom.

Product	Feedstock	Electron requirement (mole e ⁻ mole product ⁻¹)	Production (kg d ⁻¹)	Value (€ d ⁻¹)	Permissible system cost (€ m ⁻²)	CO ₂ fixed (kg d ⁻¹)
Ethanol	CO ₂	12	2.8	1.7	-	6.9
Butanol	CO ₂	24	2.2	3.2	17	11.8
Acetate	CO ₂	8	5.3	3.4	21	10.3
Citrate	CO ₂	18	7.7	7.5	112	6.9
Succinate	CO ₂	14	6.0	11.3	196	7.7
	fumarate	2	41.9	79.3	1684	NA
1,3-propanediol	CO ₂	16	3.4	4.6	47	13.7
	Glycerol	2	34.0	36.4	744	NA
Caproate	Acetate	24	3.4	13.0	232	NA

- : negative outcome

5 NA: Not Applicable

6 Figures

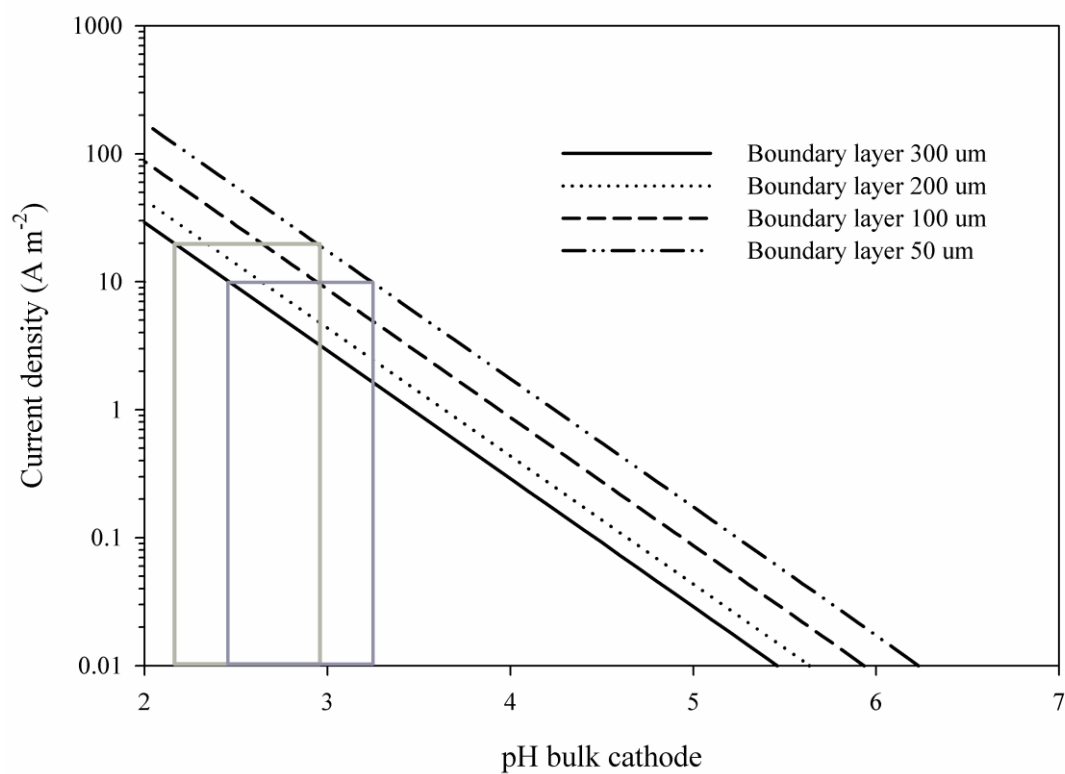


Figure 1. Attainable current densities in function of the pH gradient between the cathode surface (fixed at pH 8) and the bulk phase, based on a simple proton diffusion model. Rectangles indicate required cathode bulk pH to attain 10-20 A m⁻², and is function of the thickness of the hydrodynamic boundary layer.